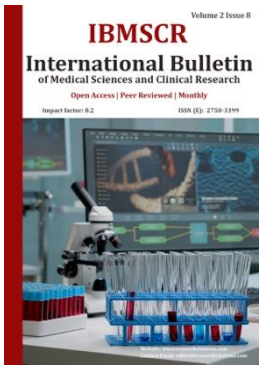

THE IMPORTANCE OF THE GUT AND VAGINAL MICROBIOME IN THE CONTEXT OF PREPARATION FOR IN VITRO FERTILIZATION PROGRAMS

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Annotation. The sum total of all microorganisms that live in association with humans constitutes the human microbiome [1]. In dysbiotic conditions, a significant role is given to microorganisms that inhabit the entire genital tract, and they also have the potential to affect the success of conception and delivery. Environmental factors, a combination of prenatal, postnatal and intrapartum factors through the microbiome, influence all stages of a woman's life [3,5,7]. The ability to conceive and carry a pregnancy to term is largely due to the interaction of a woman's macroorganism with microorganisms involved in the formation of the microbiome of the intestine, vagina and other organs [1,2,8]. Until recently, the microbiome of the female reproductive system: as the uterine cavity, fallopian tubes, ovaries, mammary glands were considered absolutely sterile. According to modern authors it is established that in the female genital tract there is an ecological niche with a specific population of microorganisms. The microbial-tissue complex of the female genital tract being a part of a unified system of mucous membranes: gastrointestinal tract (GIT), respiratory tract, urinary system, etc., quickly responds to metabolic disorders of the organism itself and to dysbiotic changes, primarily in the GIT as the main reservoir of microbiome under the external influence of the ecosystem [4,8].

Keywords: vaginal microbiome, macroorganism, microorganism, IVF

It is well known that attempts to treat infertility using in vitro fertilization (IVF) are not always successful. In an attempt to determine the causes of unsuccessful IVF attempts, many reproductologists have begun to pay great attention to the state of the endometrium and the nature of the uterine cavity microbiome. A number of studies have established an increase in the types of pathogens, such as Gardnerella, streptococci, Escherichia coli, shigella. Moreover, microflora peculiar to the intestine (E. coli, shigella, etc.) are present in the vaginal microbiome [3,4,10,11]. At present, it is absolutely proven that disruption of the intestinal and vaginal microbiome is accompanied by a pronounced cytokine imbalance [8,9,13].

The underlying causes leading to unsuccessful IVF infertility treatment attempts are still not fully understood. It is likely that disturbances in the vaginal and intestinal microbiome can significantly reduce the effectiveness of IVF programs. At the same time, the study of the microbiome may contribute to the pathogenetic justification of rational ways of ante- and intrapartum fetal protection and prevention of gestational complications. The aim of our work was to study the vaginal and intestinal microbiota in women with infertility in preparation for IVF programs.

Materials and methods of research. Forty women suffering from infertility were examined. Primary infertility was in 12 and secondary infertility in 28 patients. The 1st group

consisted of 20 women suffering from infertility, who had 2-4 unsuccessful IVF attempts in their anamnesis; the 2nd group (comparison) consisted of 20 women who had pregnancy after infertility treatment and ended in premature labor. The control group consisted of 20 healthy women.

Bacteriologic studies of vaginal and intestinal microbiome were performed by light microscopy. Content from the posterior vaginal vault was taken with a sterile swab Copan innovation (Italy) before vaginal examination. The material was delivered to the laboratory within 1 hour, or placed in a tube with buffer and delivered overnight. Serial dilutions were prepared in the laboratory in tubes at a rate of 1:10. Seeding was performed on a number of nutrient media, allowing the detection of the maximum possible spectrum of microorganisms. Dry commercial nutrient media were used for cultivation. Counting of colony-forming units (CFU) and conversion to 1 ml of biomaterial (CFU/ml) was performed. Microorganisms were cultured in a thermostat at 370C, yeast-like fungi at 220C. Pure cultures were isolated on 5% blood agar, yolk-salt agar, Endo and Sabouraud media. Identification of isolated bacteria was performed by morphological, tinctorial, cultural and biochemical characteristics using entero-, staphylo-, anaerotests (Lachema, Czech Republic).

Bacteriologic examination of feces as well as evaluation of colonic microflora was performed in accordance with the industry standard [6]. Stool was collected with a swab placed in a sterile tube and sent to the bacteriological laboratory no later than 1-2 hours after collection. Counting of colony-forming units (CFU) and conversion to 1 g of biomaterial (CFU/g) was performed.

The level of proinflammatory interleukins: interleukin-1 (IL-1ß), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor (TNF-α) and anti-inflammatory interleukins: interleukin-4 (IL-4), interleukin-10 (IL-10) in serum was determined. The studies were performed on an immunoenzyme analyzer of Shanghai Kehua Laboratorium System Co.Ltd; KHBst-360 using a set of test systems "Vector-Best" (ZAO, Russia). The technique is based on the solid-phase "sandwich" variant of immunoenzyme analysis using mono- and polyclonal antibodies sorbed on the surface of wells of a collapsible polystyrene tablet.

Statistical processing of the obtained results was carried out on a Pentium-IV personal computer using Microsoft Office Excel-2012 software package, including the use of built-in functions of statistical processing.

Results and Discussion. The age of the women was 27.0±2.5 years. The duration of infertility was from 3 to 6 years. In patients of group 1 and 2, the frequency of chronic adnexitis was 87.5% (35), vaginal dysbiosis - 77.5% (31), inflammatory diseases of the cervix - 47.5% (19). Also, there was chronic tonsillitis in 37.5% (15) and chronic pyelonephritis in 57.5% (23). In infertile patients, obstruction of fallopian tubes occurred in 27 (67.5%). After laparoscopic surgeries, patency was restored in 16 of them.

The study of vaginal microflora revealed that in women of the 1st and 2nd groups, lactobacilli (lg 3.42±0.29*) and bifidobacteria (lg 3.8±0.51) prevailed. But their content was significantly ($p<0.01$) inferior to similar indicators of the control group. (table 1).

Table 1

Microorganisms	Group 1 n =20	Group 2, n=20	Control group , n=20

	%	lg M±m КОЕ/мл	%	lg M±m КОЕ/мл	%	lg M±m КОЕ/мл
bifidum	50,0	2,6±0,4	62,5	3,8±0,51	85	4,60±0,47
Corynebacterium sp.	15,0	1,11±0,21	20,0	1,21±0,41	25	1,35±0,54
Lactobacillus	60,0	3,08±0,23*	70,0	3,42±0,29*	95	5,46±0,33
E.coli ЛП	40,0	1,76±0,35	27,5	1,68±0,45	25	1,63±0,65
E.coli ЛН	45,0	2,6±0,28*	40,0	2,16 ±0,39*	10	0,76±0,52
	25,0	1,26±0,16*	20,0	1,16±0,36*	-	-
Eubacterium sp.	45,0	1,48±0,32	40,0	1,44±0,36	35	1,63±0,58
sp.	50,0	3,3±0,54*	47,5	3,03±0,44*	20	1,44±0,58
sp.	30,0	2,46±0,44	25,0	1,76±0,39	25	1,37±0,54

Characterization of the vaginal microbiome

However, E.coli LP (lg 1.76±0.35), E.coli LN (lg 2, 6 ±0.28) and S. aureus (lg 1.26 ±0.16), which were significantly higher than those of healthy women, were the most frequently isolated in women of these groups, while S. aureus was not detected at all in the control group.

Of the Enterobacteriaceae family, only E. coli (20%, lg 3.3 ± 2.3 CFU/mL) was registered in the vagina. E.coli LN values with detection rates ranging from 10% to 45% tended to replace the opportunistic flora. Eubacterium, as well as Peptostreptococcus ¬¬ were in reduced numbers (p<0,05). Thus, according to our studies, the laboratory picture fits into moderate vaginal dysbiosis.

Similar studies of the intestinal microflora in infertile patients (Table 2) showed that there was a slight decrease in the detection rate of Lactobacillus rr. (75%) and Bifidobacterium (70%), with a concomitant increase in Peptostreptococcus sp. (45%) and Bacteroides sp. (95%) compared to the control group of women.

Table 2

Characterization of the gut microbiome

Microorganisms	Group 1 n =20		Group 2, n=20		Control group , n=20	
	%	lg M±m КОЕ/г	%	lg M±m КОЕ/г	%	lg M±m КОЕ/г
Lactobacillus sp.	60,0	3,22±0,42*	75,0	5,44±0,6*	100,0	7,89±0,12
Bifidobacterium sp.	60,0	3,27±0,47*	70,0	4,37±0,6*	100,0	7,82±0,15
Bacteroides sp.	95,0	3,07±0,79*	90,0	2,17±0,6*	85,0	1,16±0,63
Peptostreptococcus sp.	45,0	6,37±0,19	40,0	4,16±0,8	15,0	7,53±0,58

E. coli ЛП	80,0	6,26±0,02*	85,0	7,96±0,1*	100,0	8,72±0,19
E. coli ЛН	65,0	4,94±0,35*	50,0	3,76±0,2*	20,0	7,38±0,56
Proteus sp.	40,0	4,98±0,86*	35,0	3,76±0,6*	15,0	1,49±0,68
Klebsiella sp.	15,0	2,46±0,70*	15,0	2,46±0,7*	10,0	0,99±0,50
S. epidermidis	30,0	0,70±0,40	35,0	0,90±0,50	75,0	0,49±0,34
S. aureus	45,0	1,85±0,52*	30,0	1,60±0,3*	10,0	3,19±0,54
Streptococcus gr.A	40,0	3,20±0,63*	30,0	1,60±0,3*	10,0	0,55±0,27
Streptococcus gr.D	60,0	0,86±0,38*	65,0	0,72±0,2*	90,0	0,48±0,01
Candida albicans	45,0	3,01±0,47*	40,0	3,72±0,*	20,0	5,64±0,36

There was also a significant increase in the isolation of E.coli LN (65%) and Proteus sp. (40%), Staphylococcus aureus (45%).

In the control group of women in the fecal bacteriologic examination of feces, only 10% (2) had a disorder of the intestinal microbiome. The content of bifido- and lactobacilli was significantly reduced ($p<0.05$) compared to formally normal. Among all representatives of facultative microflora, Klebsiella and Protea were significantly higher than the norm ($p<0.05$). Other representatives of facultative and obligate groups by frequency of detection and quantitative characteristics also corresponded to the norm. The data of our study indicate the presence of intestinal dysbiosis in the patients of the studied groups.

Thus, the results of bacteriological examination of patients with infertility showed that the vaginal microbiome is characterized by a sharp decrease in normoflora (lacto-, bifidobacteria) and a significant increase in opportunistic flora (eubacteria, Prevotellae, peptostreptococci).

In the intestinal microbiome there was found a decrease in species diversity, an increase in the number of enterococci, Klebsiella, proteobacteria, characteristic for intestinal dysbiosis associated with inflammation. Thus, in the patients of the 1st and 2nd groups, a violation of the vaginal microbiome was found in 71.7%, and of the intestinal microbiome - in 86.7% of patients. However, changes in the intestinal microbiome in healthy women occurred in 10% of women.

When studying the level of cytokines in serum, it was found (Table 3) that in women of the control group the level of cytokine IL-1β in serum was 2.35±0.18 pg/mL, IL-2 - 7.54±0.64 pg/mL, IL-4 -5.76±0.44 pg/mL.

The serum IL-6 content was 2.25± 0.17 pg/mL, IL-8 was 6.36± 0.58pg/mL, IL-10- 23.14± 1.57 pg/mL, and TNF-α level was within 1.68± 0.13 pg/mL.

Table 3

Cytokine parameters in blood serum of patients with infertility

Indicator pg/mL	Control group (n=20)	Women with infertility (n=40)	P

IL-1β	2,35±0,18	14,6±0,87	<0,001
IL-2	7,54±0,64	11,14±0,91	<0,01
IL-4	5,76±0,44	3,15±0,23	<0,001
IL-6	2,25±0,17	4,83±0,39	<0,001
IL-8	6,36±0,58	9,98±0,63	<0,001
IL-10	23,14±1,57	7,36±0,62	<0,001
TNF-a	1,68±0,13	3,12±0,28	<0,001

Analysis of cytokine parameters in patients with infertility revealed a significant increase in serum IL-1β production by 6.2 times (14.6±0.87 pg/mL), p<0.05. IL-1 is an inducible protein whose synthesis is essential for the acute-phase response. The main producing cells are monocytes, macrophages, endothelium and other cells. Excessively high levels of IL-1 indicate the possibility of undesirable immunopathologic processes. IL-1 is characterized by its ability to stimulate the production of prostaglandins. Keeping this cytokine at a low level is one of the factors contributing to the preservation of pregnancy.

Also, in patients with infertility the IL-8 level was increased 1.6 times (9.98±0.63 pg/mL) compared to the control group (p<0.05). The high level of spontaneous IL-8 production may indicate a significant activation of mononuclear phagocytes-producers of proinflammatory cytokines, which play an important role in the development of immunopathologic processes.

The obtained data on the increase in IL-1β and IL-8 are a reflection of the activity of the inflammatory process. The increased concentration of proinflammatory cytokines indicates that in this contingent of patients the inflammatory reaction has a systemic character.

As the results of our study show, in infertile women there is a 2.1-fold increase in serum IL- 6 (4.83±0.39 pg/mL) compared with healthy women (P<0.05).

In addition, in women with infertility, serum TNF-α levels increased 1.9-fold (3.12±0.28 pg/mL) compared with those of controls (P<0.05)). TNF-α is formed by tissue macrophages, monocytes and lymphocytes in the zone of acute inflammation, enhances the basic functions of leukocytes, stimulates histamine release by basophils and mast cells, causes activation of fibroblasts, smooth myocytes and vascular endothelium in the focus of inflammation, induces synthesis of proteins of the acute phase of inflammation. TNF-α hypersecretion leads to a significant increase in the number of apoptotic trophoblast cells, which may be one of the factors contributing to pregnancy failure.

In our study, anti-inflammatory cytokines were: IL-4 - 3.15±0.23 pg/mL (P<0.05), IL-10 - 7.36±0.62 pg/mL (P<0.001), which is 1.5 times and 3.1 times lower than similar parameters of the control group, respectively.

We determined the sensitivity and specificity of cytokines. It was found that the most pronounced sensitivity and specificity were IL-6 and IL-10. Thus, the sensitivity of IL-6 - 82,6%, IL-10 -89,6% and specificity: 88,9% and 90,0% respectively.

Thus, the results of our study allow us to assert that the study of cytokine balance is significant for assessing the directionality of the immune response. Perhaps, it may be reflected in the ability of women to conceive.

It should be noted that the studies indicate a certain relationship between the state of the vaginal and intestinal microbiome. Violation of the vaginal and intestinal microbiome is a factor affecting the balance of pro-inflammatory and anti-inflammatory cytokines, which indicates the development of a systemic inflammatory response in the woman's body and is undoubtedly a factor that disrupts the processes of conception.

Conclusions. Thus, the results of our study allow us to state that the risk factors for failed IVF attempts and premature births are disorders of the vaginal (71.7%) and intestinal (86.7%) microbiome % in women with infertility.

The increase in the level of pro-inflammatory cytokine IL-6 in 2.1 times and decrease in anti-inflammatory cytokine IL-10 in 3.1 times indicates the development of a systemic inflammatory response, which may be reflected in the possibility of conception and pregnancy.

In order to develop individual infertility treatment programs, it is necessary to include in the examination the assessment of the vaginal and intestinal microbiome and, accordingly, to carry out the correction of these disorders.

In preparation of patients for IVF programs, as well as in pregravidar preparation of patients with infertility and premature births in the anamnesis should be included in the treatment regimens of pre- and probiotics, which will contribute to improving the effectiveness of IVF programs and reduce reproductive losses.

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